BIOSCAVENGERS AS A PRETREATMENT FOR NERVE AGENT EXPOSURE

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The use a bioscavenger has emerged as a new approach to reduce the in vivo toxicity of chemical warfare nerve agents. As an improvement of over current treatment, a biological scavenger should have no or minimal behavioral or physiological side effects, should provide protection up to a 5 LD50 exposure and should be devoid of any behavioral or physiological side effects. Studies with equine or human butyrylcholinesterase or fetal bovine serum acetylcholinesterase showed that none of these scavengers exhibited behavioral side effects when given alone to rats or monkeys. Furthermore, each was capable of providing protection against 2 to 16 LD50s of GD, GB or VX depending on the scavenger and the test species. The results to date support the value of this approach as the next generation of pharmaceuticals to afford protection against nerve agent poisoning.

The conventional approach to treatment of organophosphorus (OP) intoxication involves efforts to counteract the effects of acetylcholinesterase (AChE) inhibition. Cholinolytic drugs such as atropine are administered at the onset of signs of OP intoxication to antagonize the effects of the elevated acetylcholine levels that result from the inhibition of AChE (1). Additionally, an oxime nucleophile is given, which reacts with the inhibited (phosphonylated) enzyme to displace the phosphonyl group and restore normal activity (2). In the United States, the oxime of choice for treatment of nerve agent poisoning is the chloride salt of 2-PAM, usually referred to as 2-PAM Cl, although bis-pyridinium oximes may be more effective depending on the particular organophosphorus agent (3). Anticonvulsant drugs such as diazepam are also administered to control OP-induced tremors and convulsions. In conjunction with therapy, individuals at high risk for exposure to nerve agents are pretreated with a spontaneously reactivating AChE inhibitor such as pyridostigmine, which temporarily masks the active site of a fraction of AChE molecules and thus protects the enzyme from irreversible inhibition by the OP agent (4). Several nerve agents, including GF, sarin, and in particular soman, present an additional therapeutic challenge in that after they inhibit AChE, they undergo a second reaction in which the phosphonyl group attached to the inhibited enzyme is dealkylated. This process, known as aging, results in a phosphonylated AChE that is refractory to either spontaneous or oxime-mediated reactivation (5). The ineffectiveness of therapeutically administered oxime as a treatment for some nerve agents explains the continued research efforts aimed at alternative approaches to protection (6).

In contrast, recent efforts have focused on identifying proteins that can act as biological scavengers of organophosphorus compounds and can remain stable in circulation for long periods of time. This approach avoids the side effects associated with current antidotes (6, 7-14) and the requirement for their rapid administration, by prophylactically inactivating (through sequestration or hydrolysis) anticholinesterase agents before they can react with the target AChE. The time frame for this inactivation

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to occur before endogenous AChE is affected is quite narrow (estimated to be approximately two minutes in humans (15). so especially for situations involving acute exposure, the scavenger function must be very rapid, irreversible and specific. Ideally, the scavenger would enjoy a long residence time in the bloodstream, would be biologically innocuous in the absence of nerve agent and would not present an antigenic challenge to the immune system and efforts have focused on enzymes of mammalian (usually human) origin.

Candidate bioscavenger proteins, in general, function either by stoichiometricly binding and sequestering the anticholinesterase or by catalytically cleaving the OP substrate into biologically inert products. In the former category are naturally occurring human proteins that bind nerve agents, including enzymes such as cholinesterases (ChEs) and carboxylesterases (CaEs), as well as antibodies specific for nerve agent haptens. Each of these stoichiometric scavengers has the capacity to bind one or two molecules of nerve agent per molecule of protein scavenger. While this approach has been proven to be effective in laboratory animals, it has the disadvantage that the extent of protection is directly proportional to the concentration of unexposed, active scavenger in the bloodstream at the time of nerve agent exposure.

Candidate enzymes with *bona fide* catalytic activity against nerve agents include the human organophosphorus acid anhydride hydrolases (OPAHs), such as paraoxonase (hu-Pon). Additionally, the ability to generate catalytic antibodies in response to appropriate transition state analogs (16,17) suggests that nerve agent-specific antibodies that catalyze hydrolysis of their ligands could be effective bioscavengers. Finally, the ability to engineer site-specific amino acid mutations into naturally occurring scavenger enzymes can allow investigators to alter the binding and/or catalytic activities of these enzymes. In general, the use of scavengers with catalytic activity would be advantageous because small amounts of enzyme, meaning lower concentrations in circulation, would be sufficient to detoxify both large amounts of nerve agent.

To be an improvement over the existing therapeutic approach for providing protection against nerve agent poisoning, a biological scavenger, either stoichiometric or catalytic, should satisfy three critical criteria. First it must be safe, producing no untoward effects in its own right. Secondly, it must provide an increase in efficacy or equal efficacy with no need for additional drugs, e.g., is easier to administer and is less time dependent in administration. Finally, it must produce an efficacious response with reduced level of behavioral or physiological incapacitation. Considerable effort has been made in the past five years in this area and currently there are a variety of proteins that meet these criteria

BEHAVIORAL EFFECTS OF SCAVENGERS ALONE

Several studies have examined the behavioral effects of the biological scavengers themselves in the absence of cholinesterase inhibitors (Table 1). Genovese and Doctor (18) reported that rats were trained to perform a passive avoidance task, a motor activity, and a scheduled-controlled behavior. The performance of animals before and after administration of purified equine-butyrylcholinesterase (eq-BuChE) at a dose that would be expected to provide protection against an exposure of several LD_{50}s of an organophosphorus compound was assessed. In all cases, the authors report that eq-BuChE did not disrupt performance of any of the learned tasks, did not upset the circadian cycle of light/dark activity, and had no effect on motor activity. These outcomes were in contrast to those observed when the standard cholinolytic, atropine, was administered. In a separate study also using eq-BuChE (19), rhesus monkeys were trained to perform a serial probe recognition (SPR) task. Once the animals became proficient at the task they received eq-BuChE in a dose similar to that reported by Broomfield et al. (20) as sufficient to afford protection against a 2 or 3 LD_{50} soman challenge. The authors reported that repeated administration of commercially prepared eq-BuChE had no effect on the behavior of the monkeys as measured by the SPR studies. Given the lack of behavioral effects and the relatively long *in vivo* half-life of the eq-BuChE, they concluded that this biological scavenger was potentially more effective than current chemotherapeutic treatments for organophosphorus intoxication.

TABLE 1. Protection from Organophosphorus Intoxication by Stoichiometric Bioscavengers.

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Serum $T_{1/2}$[b]	Reference
FBS-AChE	Rhesus Monkey	GD	2-5	30-40 Hrs	28, 29
"	Mouse	GD	2 (w/ Atropine + 2-PAM)	40-50 Hrs	24
"	"	GD	2 (after CBDP treatment)	~24 Hrs	30
"	"	GD	2-8	24-26 Hrs	21, 22, 26
"	"	MEPQ	4	~24 Hrs	22, 30
"	"	VX	2-3.6	~24-50 Hrs	24, 30
					22
eq-BuChE	Rhesus Monkey	GB	1	620 Hrs	20
"	"	GD	2 (4 w/ atropine)	"	20
"	"	GD	5	30-40 Hrs	29
hu-BuChE	Rhesus Monkey	GD	2	~30 Hrs	32
"	"	VX	1.5	"	32
"	Rat	GD	2-3	46 Hrs	23
"	"	VX	2	"	23
"	Mouse	GD	2.1	21 Hrs	32
"	"	GB	1.6	"	32
"	"	GA	1.8	"	32
"	"	VX	4.9	"	32
CaE[c]	Mouse	GD	16	N.D.[d]	37
"	Guinea Pig	GD	3.5	N.D.	37
"	Rabbit	GD	3	N.D.	37
"	Rat	GD	8-9	N.D.	28, 37
"	"	GB	8	N.D.	37
"	"	GA	4-5	N.D.	37
"	"	VX	1	N.D.	37
"	"	Paraoxon	2	N.D.	37

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after scavenger administration. [b] Half-life of scavenger in blood circulation. [c] For each s the activity of the host's endogenous CaE was tested. [d] Not determined. Because CaE is an endogenous serum protein, the protection it offers was measured by comparing L values in untreated and CBDP-treated animals; 2 mg/kg CBDP completely abolishes endogenous CaE activity (28).

BEHAVIORAL EFFECTS OF SCAVENGER AND NERVE AGENT EXPOSURE

Studies on the behavioral effects that result from nerve agent exposure of animals pretreated with biological scavengers tend to include studies in both rodents and non-human primates (Table 2). The rodent data are, for the most part, limited to observations of animals after the experimental procedure (21-23) or to the ability of mice to respond to an inverted screen test (24, 25). Maxwell and co-workers (26) applied a mixture of tests including the inverted screen test as well as activity and motor function assays to mice given fetal bovine serum-AChE (FBS-AChE). In all of these studies, the authors report that animals pretreated with a scavenger, such as FBS-AChE, eq-BuChE, or human-BuChE (hu-BuChE), followed by exposure to soman or VX, exhibited no deficits in behavior. Animals that received no pretreatment all suffered notable impairment, and the time for recovery was on the order of days or longer. Brandeis et al. (27) examined the effects of soman on rats that either had or had not been pretreated with hu-BuChE and trained to perform the Morris water maze behavioral task. Rats given a sub-lethal dose of soman alone had significant impairments in cognitive function that manifested itself over a period of several weeks. Pretreatment with hu-BuChE provided substantial protection from these behavioral decrements; the performance of hu-BuChE-pretreated, soman-exposed rats was indistinguishable from that of control rats exposed only to saline. The authors also observed that rats administered the scavenger in the absence of soman were devoid of impairments in behavioral performance.

Studies in non-human primates have led to similar conclusions. Broomfield et al. (20) and Maxwell et al. (28), studying eq-BuChE and FBS-AChE respectively as scavengers, used the SPR task to evaluate the ability of these two scavengers to protect against behavioral decrements after soman poisoning. After eq-BuChE pretreatment followed by 2 LD_{50}s of soman, animals exhibited a transient performance decrement at 8 hours post-challenge. Thereafter they returned to baseline performance levels and were followed for up to six days. A related study in rhesus monkeys trained to perform a Primate Equilibrium Platform (PEP) task detected no performance decrements in animals given FBS-AChE alone, or pretreated with scavenger prior to a cumulative challenge of 4 LD_{50}s of soman. When eq-BuChE was the scavenger, transient performance decrements were observed when the soman challenge exceeded a cumulative dose of 4 LD_{50}s, although all of the animals survived this otherwise lethal dose. In no case were residual or delayed performance effects detected up to six weeks after nerve agent exposure in animals pretreated with either cholinesterase (29). A summary of the results from these primate studies can be found in Doctor et al. (30, 31).

Raveh and co-workers (32) described the protective effects of hu-BuChE in rhesus monkeys exposed to soman or VX. The monkeys were first trained on a spatial discrimination (SD) task and then pretreated with hu-BuChE before exposure to the organophosphorus nerve agents. The scavenger afforded protection against the lethality of soman or VX, but with respect to protection against behavioral deficits, the results were mixed. Despite less than ideal protection against performance decrements, the authors concluded that the hu-BuChE scavenger offered a high level of protection against soman-induced behavioral deficits. They also commented on the consistency of results across species for this type of pretreatment, and suggest that it should be possible to predict the extent of protection that would be afforded humans based on their own results (32) and the work of others.

BEHAVIORAL EFFECTS OF SCAVENGERS VERSUS CONVENTIONAL THERAPY POST EXPOSURE

Results reported using the SPR task as a measure of behavioral performance in non-human primates when scavenger pretreatment was followed by administration of multiple LD_{50}s of soman (20, 28, 33) can be compared with similar studies using conventional therapy (Table 2). Pyridostigmine pretreatment alone had no effect on SPR performance in trained animals. Following soman exposure and treatment with atropine and oxime either with or without diazepam, recovery of pre-exposure performance on the SPR task took from six (when co-administered diazepam) to 15 days (without the anti-convulsant; 34) The authors concluded that diazepam would be an excellent adjunct to the pyridostigmine pretreatment/atropine, oxime treatment regimen. The prolonged recovery time of six to 15 days after conventional therapy contrasts

dramatically with the results from bioscavenger prophylaxis. The lack of or presence of only a subtle, transient decrease in SPR performance, when a bioscavenger is used as a pretreatment (20, 28), offers impressive evidence for the value of this approach as affording protection against behavioral effects following nerve agent poisoning.

CONCLUSION

Organophosphorus nerve agents represent a very real threat not only to war fighters in the field but also to the public at large (35). Nerve agents have already been used by terrorist groups against a civilian population and, due to their low cost and relative ease of synthesis, are likely to be used again in the future (36). Current therapeutic regimes for nerve agent exposure are generally effective at preventing fatalities if administered in an appropriate time frame. Using bioscavengers would provide a capability for extended protection against a wide spectrum of nerve agents and would eliminate the need for extensive postexposure therapy. Work is currently underway to isolate gram quantities of BChE for safety and efficacy testing. The completion of this concept exploration phase should provide the data required for a program decision review pursuant to filing for an investigational new drug application.

TABLE 2. Protection from Behavioral Deficits by Bioscavengers or Conventional Therapy.

Protection	Test Species	Toxin	Dose (LD_{50})[a]	Behavioral Test(s)	Impairment[b]	Reference
Without Nerve Agents:						
Atropine	Rat	None	0	Passive Avoidance, VI56 s Schedule	Total	18
eq-BuChE	"	"	"	Passive Avoidance, Motor Activity, VI56 s Schedule	None	18
hu-BuChE	"	"	"	Morris Water Maze	None	27
Pyridostigmine	Rhesus Monkey	"	"	Primate Equilibrium Platform (PEP)	Substantial	38
eq-BuChE	"	"	"	Serial Probe Recognition (SPR)	None	19
"	"	"	"	Observation, SPR	Subtle SPR defect	33
"	"	"	"	"	None	19
hu-BuChE	"	"	"	Spatial Discrimination	Minor (1/4 had errors)	32
With Nerve Agents:						
Pyridostigmine & Atropine	Mouse	GD	8	Inverted Screen, Motor Function, Lacrimation, & Activity Level	Near Total	26
HI-6 & Atropine	"	GD	8	"	Near Total	"
FBS-AChE	"	GD	8	"	Very Minor	"
"	"	VX	2-3	Inverted Screen	Minor (1/10 failed)	24
PTE[e]	"	GD	3-4	"	None	25
eq-BuChE	Rat	MEPQ	> 1	VI56 s Schedule	Moderate (70% of control)[f]	18
hu-BuChE	"	GD	1.5	Observation	Minor (tremors in 1/6)	23
"	"	VX	1.4	"	None	23.
"	"	GD	1.5	Morris Water Maze	None	27
FBS-AChE	Rhesus Monkey	GD	5	Observation, PEP	None	29
"	"	GD	2.7	Observation, SPR	None	28
eq-BuChE	"	GD	2	"	Transient	20
"	"	GD	2	"	Subtle SPR defect[g]	33
eq-BuChE	"	GD	4	Observation, PEP	None	29
hu-BuChE	"	GD	3.3	Spatial Discrimination	Minor (1/4 had errors)	32
Pyridostigmine	"	GD	~0.4	Observation, PEP	Substantial	38
Pyridostigmine, Atropine & 2-PAM	"	GD	2	Observation, SPR	Substantial	20
"	"	GD	5	"	Substantial	34
Pyridostigmine, Atropine, 2-PAM & Diazepam	"	GD	5	"	Substantial	34

[a] Median lethal dose of nerve agent administered. [b] Behavioral impairment relative to untreated animals. [c] Not Determined [d] Phosphotriesterase from *Pseudomonas diminu*
[e] The VI56 s behavior is a food-reward based task. The authors speculate that nausea caused by nerve agent exposure, rather than a cognitive deficit, may have caused the behavioral impairment. [f] The sustained subtle defect was in addition to the short-term, substantial defect described by Broomfield, et al. (20)

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